

COMPLEX PATTERNS OF PHENOTYPIC PLASTICITY: INTERACTIVE EFFECTS OF TEMPERATURE DURING REARING AND OVIPOSITION

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Abstract. Temperature profoundly affects growth and life history traits in ectothermic animals through selection (i.e., genetic) and through direct effects on the phenotype (i.e., nongenetic/plasticity). We examined the effects of rearing temperature (24°, 30°, and 36°C) on adult body size and development time and the interactive effects of temperature experienced during rearing and oviposition on several life history traits (age-at-first-reproduction, fecundity, egg size, egg development, and egg hatching) in two populations of the seed beetle, *Stator limbatus*, collected at different elevations. The higher elevation population was larger and matured sooner than the low-elevation population when raised at the lower temperature, but the reverse was true at the higher temperature suggesting that these populations have adapted to local temperature. There were interactions between the effects of rearing temperature and oviposition temperature for age-at-first-reproduction, fecundity, egg development, egg hatching, and two composite measures of fitness, generating complex reaction norms. The most dramatic example of this was a large maternal effect on egg hatching; females raised at low temperature produced eggs that had substantially reduced hatching when laid at high temperature. Our experimental design also allowed us to explore the adaptive significance of acclimation. Beetles reared at intermediate or low temperature had the highest fitness at multiple oviposition temperatures. There was little support for the “beneficial acclimation hypothesis,” which predicts that beetles should have higher fitness at the temperature at which they were reared; acclimation had only a small effect on fitness. This study shows that temperature-mediated plasticity can be complex, but these complex patterns can yield new insights into the evolution of phenotypic plasticity.

Key words: acclimation; adaptation; beneficial acclimation hypothesis; body size; maternal effects; phenotypic plasticity; *Stator limbatus*; temperature.

INTRODUCTION

For many ectothermic animals, the temperature experienced during development strongly affects many growth and life history traits. For instance, body size (Atkinson 1994), development time (Atkinson 1994), and egg/offspring size (Fox and Czesak 2000) generally increase with decreasing temperature, whereas fecundity (e.g., Fischer et al. 2003a) generally declines with decreasing temperature. These phenotypic responses may be a result of adaptation to different thermal environments or may be an unavoidable consequence of a temperature effect on the organism's physiology during development (developmental plasticity). The extent to which temperature-mediated natural selection vs. the environmental effect of temperature differentially affect traits at different temperatures has generated debate between adaptive (genetic) and developmental (nongenetic) hypotheses (Van Voorhies 1996, 1997, Mousseau 1997, Partridge and Coyne 1997).

Evidence for genetic adaptation to temperature comes from studies of patterns of geographic variation

in nature. Body size and egg size of ectotherms exhibit clinal variation along latitudinal (e.g., Lonsdale and Levinton 1985, Armbruster et al. 2001) and altitudinal gradients (e.g., Berven 1982). When individuals are reared in a common environment, these differences are usually found to be partially genetically based. In *Drosophila melanogaster*, body size clines are often repeatable across continents (e.g., James et al. 1995, Van't Land et al. 1995). These clines can evolve rapidly, only a couple of decades after introduction to a new continent (Huey et al. 2000), indicating strong natural selection on either body size, egg size, or some correlated trait. Laboratory natural selection experiments, in which insects are allowed to adapt to high or low temperature, also demonstrate that both body size and egg size evolve to be larger at colder temperatures (Partridge et al. 1994, Azevedo et al. 1996; but see Santos et al. 2004).

Nongenetic responses to developmental temperature (phenotypic plasticity) are widespread. For example, growth at lower temperature results in increased adult body size in >80% of ectothermic animals (Atkinson 1994), and many arthropods typically lay larger eggs at lower temperatures (Fox and Czesak 2000). Nongenetic responses to developmental temperature are thus very similar to those produced by evolution at

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different temperatures making it difficult to distinguish between genetic (evolution due to selection) and nongenetic (plasticity) causes for geographic patterns. Common garden and reciprocal transplant experiments are required to disentangle these factors.

Adding to this complexity, patterns of phenotypic plasticity may change if the temperature encountered in an earlier vs. a later life stage interact to affect a phenotype. For instance, immature stages of many insects develop at different times of the year and in other physical localities than adults, subjecting these life stages to different thermal environments. Also, immatures are less mobile than adults and are often unable to leave unfavorable thermal environments. Nevertheless, few studies have considered both the separate and interactive effects of temperature experienced during immature development (rearing) vs. adult (oviposition) stages of the life cycle (Fox and Czesak 2000). We need to understand how these interactions affect reaction norm shape to predict response to selection in more complex environments.

Studies on the effects of temperature experienced at an early and later life stage provide a unique opportunity to study the adaptive significance of acclimation (a nongenetic, physiological alteration in a phenotype caused by a change in the environment). It is commonly assumed that development at a certain temperature will enhance fitness in the adult stage at that temperature (“beneficial acclimation hypothesis”; Leroi et al. 1994). Alternatively, acclimation may be nonadaptive if rearing in one environment enhances fitness in alternate environments to which the organism is not acclimated (Huey et al. 1999). For example, development at an intermediate (“optimal developmental temperature hypothesis”), low (“colder is better hypothesis”), or high (“hotter is better hypothesis”) temperature may enhance fitness in a variety of adult thermal environments. Recently, factorial designs have been developed to explore the adaptive significance of acclimation (Huey and Berrigan 1996). These designs and recently developed statistical models allow for several competing hypotheses to be tested simultaneously (Huey et al. 1999). Assessments using this approach have shown that while the beneficial acclimation hypothesis is supported in some cases, alternative hypotheses often have a greater influence on fitness (Huey et al. 1999, Gibert et al. 2001). However, these studies have been criticized as having examined the adaptive significance of developmental plasticity (irreversible as well as reversible changes to the phenotype induced during development) instead of traditionally studied acclimation (reversible changes to the phenotype induced in later life stages) (Wilson and Franklin 2002). Accordingly, the adaptive significance of acclimation and how it can be studied is the subject of substantial debate.

Here, we use the seed beetle, *Stator limbatus* (Coleoptera: Chrysomelidae: Bruchinae), to investigate both genetic and nongenetic responses to temperature

and the interactive effects of rearing vs. oviposition temperature and to assess the adaptive significance of acclimation. Within central Arizona, USA, *S. limbatus* experiences large daily (ranging from 24° to 39°C; mean minimum daily temperature compared to mean maximum daily temperature in Phoenix, Arizona) and spatial (25°–31°C; monthly mean in Oracle, Arizona, compared to Phoenix, Arizona; Western Regional Climate Center, Desert Research Institute) variation in temperature during August when beetles are most active. *S. limbatus* is a generalist seed parasite of legumes; adults are the only dispersing stage of the life cycle, with larvae being restricted to the seed their mother has chosen for them. Since larvae cannot move between seeds they cannot move between thermal environments.

In this study, we examine two populations of *S. limbatus* from central Arizona that occur at different elevations (Phoenix, ~500 m above sea level; Oracle, ~1400 m above sea level) and experience different local thermal environments (mean temperature difference in August of ~6°C). Our a priori expectations were that these populations are adapted to a “warm” and “cool” environment and should be genetically differentiated in a number of traits. We first assessed how temperature during rearing affects adult body size and larval development time in both populations. We then examined the separate and interactive effects of temperature experienced by beetles during rearing vs. oviposition periods of the life cycle on age-at-first-reproduction, fecundity, egg size, egg development, and egg hatching. Finally, using the approach described by Huey et al. (1999) we tested the beneficial acclimation, optimal developmental temperature, hotter is better, and colder is better hypotheses.

METHODS

Natural history of Stator limbatus

The seed beetle, *Stator limbatus* (Horn) (Coleoptera: Chrysomelidae: Bruchinae), is distributed from northern South America to the southwestern United States (Johnson and Kingsolver 1976, Johnson et al. 1989, Nilsson and Johnson 1993). It is a generalist seed herbivore that has been collected from >70 species of legumes throughout its wide geographic range, though only one or a few hosts are encountered in most locations. *S. limbatus* commonly uses *Acacia greggii* (Fabaceae: Mimosoideae), *Parkinsonia florida* (Fabaceae: Caesalpinioideae; previously *Cercidium floridum*), and *Parkinsonia microphylla* (Fabaceae: Caesalpinioideae) as hosts in central Arizona, USA. Females oviposit directly onto host seeds inside of seed pods that have either dehisced or been damaged by other organisms (e.g., mice, other bruchine beetles such as *Mimosestes* species, etc.). Upon hatching, first-instar larvae burrow into the seed directly underneath the egg, where development and pupation take place. Mating and egg

laying start ~24–48 h after emerging from the seed (in the laboratory). *S. limbatus* needs only the resources inside of a single seed to complete development and reproduce; additional food and water are not necessary.

Study populations

We used one *S. limbatus* population from Oracle, Arizona (Oracle population), and one from Phoenix, Arizona (Phoenix population). The Oracle population was collected along Highway 77 and adjacent roads in Oracle, Pinal County, Arizona, USA (32.62° N, 110.78° W; ~1400 m above sea level) in August 2000 from mature seed pods of >20 *A. greggii* trees. Mean annual temperature at this site is ~16.7°C and the August monthly mean is ~25.2°C (Western Regional Climate Center, Desert Research Institute, Reno, Nevada, USA). The Phoenix population was collected just north of Phoenix, Maricopa County, Arizona, USA (33.79° N, 112.12° W; ~500 m above sea level) in August 2000 from seed pods of >20 *P. florida* trees. Mean annual temperature at this site is ~20.9°C, and the August monthly mean is ~31.3°C. Seed pods were shipped back to the laboratory where colonies were established from >200 emerging adult beetles of each population. Colonies were maintained on *A. greggii* seeds at ~100 families each generation (at 30°C, 15:9 light : dark) prior to the experiment, which started in February 2002. Beetles were reared on *A. greggii* in the laboratory because they have high survivorship (>90%) at 30°C on this host (Fox et al. 1994).

Experimental design

We used a completely randomized design with a full 3 × 3 factorial treatment arrangement to examine the effects of rearing temperature on growth traits and the effects of rearing vs. oviposition temperature on life history traits. In brief, full-sib families (family sizes were too small to allow the use of a split brood design) of beetles from both the Oracle and Phoenix populations were assigned randomly to one of three rearing treatments (24°, 30°, or 36°C). These temperatures are within the range of temperatures normally encountered in the field (see *Introduction*). One-third of the families within each rearing treatment were randomly assigned to each of three oviposition treatments (24°, 30°, or 36°C). All progeny of a family experienced only one rearing × oviposition treatment combination; siblings were not split among the treatments. Offspring were reared in temperature-controlled growth chambers. Petri dishes were rotated daily to minimize the effects of spatial variation in temperature.

To create families, virgin males and females were paired and placed with 20 *A. greggii* seeds in a 35-mm petri dish and allowed to mate and oviposit at 30°C, 15:9 light : dark. They were checked daily for eggs. If eggs were present, seeds were scraped to one egg/seed (to eliminate larval competition) and placed in their assigned rearing treatments (24°, 30°, or 36°C, 15:9

light : dark) within 24 h of being laid ($N = 85\text{--}90$ for each rearing treatment × population combination; total = 525 families). This was repeated daily until females laid eggs on ~7–8 seeds, after which adults were discarded.

Emerging progeny were collected twice daily and development time was recorded. Beetles were weighed on an electronic balance (AT261 Delta Range; Mettler Toledo, Columbus, Ohio, USA) to the nearest 0.1 mg. Virgin beetles were placed in individual 35-mm petri dishes at their assigned oviposition treatment for 24–36 h before we allowed them to mate ($N = 24\text{--}33$ per rearing temperature × oviposition temperature × population combination; i.e., there were three rearing treatments × three oviposition treatments × two populations = 18 groups of 24–33 families). This assured that eggs laid later in the experiment had been mated at the correct oviposition temperature. Ten *A. greggii* seeds were present in the female dishes because the presence of hosts facilitates egg maturation (Savalli and Fox 2002). After 24–36 h a randomly selected virgin male originating from the same population and rearing regime was placed in the female's dish. Each dish was checked for eggs twice daily. If eggs were present, adults were transferred to a second 35-mm petri dish containing 10 *A. greggii* seeds. Females were allowed to oviposit for 24 h after which they were transferred to a third dish (60 mm) containing 30 *A. greggii* seeds upon which they were allowed to oviposit until death (to estimate lifetime fecundity).

Egg size and egg mortality were assessed by examining only eggs laid in the female's second dish because egg size is known to change over a female's life and may influence survivorship of larvae (Fox and Mousseau 1996, Fox et al. 1997). This also ensured that the eggs measured were matured at the correct oviposition temperature. Egg size of each female was determined by measuring the length of 1–3 haphazardly chosen eggs using an ocular micrometer on a 100× stereo microscope (0.005-mm precision). Measuring egg mass is not practical because removing eggs from the seed is destructive. However, egg length is highly correlated with egg mass (Fox and Mousseau 1996) and, therefore, a good estimate of overall egg size. Egg mortality was determined for each individual egg and was divided into three categories: did not develop (denoted by clear eggs), developed but did not hatch (indicated by a visible embryo), and hatched (denoted by the presence of frass in the eggs). In total 3160 offspring were reared to adult and weighed. Reproductive traits were recorded for 1372 females.

Data analyses

We analyzed all data using SAS 8.2 (SAS Institute, Cary, North Carolina, USA). Normal probability plots revealed that the data (except egg development and egg hatching) were approximately normally distributed, so no transformations were performed. We used mean trait

values of each family (approximately three offspring per family on average) in our ANOVAs (SAS PROC GLM) to remove the non-independence of siblings within treatments. For egg mortality data, we used logistic regression analysis (SAS PROC GENMOD) on individual eggs (11 756 total eggs) with family included as a covariate nested within treatment interactions to control for the variability among families on egg mortality (the family effect was highly significant for both analyses; egg development, $\chi^2_{18} = 51.44$, $P < 0.0001$; egg hatching, $\chi^2_{18} = 74.96$, $P < 0.0001$). For simplicity, results are presented by main effects and interactions although analyses for each trait were performed using a full model including main effects and all possible interactions.

We created two composite measures of fitness to assess the acclimation hypotheses. The first composite measure of fitness (r_{C1}) was the number of offspring produced by a female that survived to a first-instar larva (proportion of offspring hatching \times fecundity). The second (r_{C2}) was adjusted by the age at which each female reproduced ($r_{C1}/\text{age-at-first-reproduction}$). Normal probability plots indicated that both r_{C1} and r_{C2} were approximately normally distributed and thus no transformation was performed. Since body size and development time are affected by rearing temperature only, they are not included in our estimates of fitness. Egg size is included in fitness estimates through its effects on larval survivorship and female fecundity.

To evaluate the acclimation hypotheses, we used ANOVA with orthogonal polynomial contrasts (Huey et al. 1999). We subsampled the original data set to create the balanced design necessary for orthogonal polynomial contrasts (Sokal and Rohlf 1995). The number sampled from each treatment combination was based on the treatment combination with the lowest sample size. Separate analyses were run for each population.

We used a statistical model in which the linear (rearing_(l)) and quadratic (rearing_(q)) effects of rearing temperature, and their interactions with oviposition temperature, allowed us to assess the relative impact of each acclimation hypothesis (Huey et al. 1999). The colder is better hypothesis predicts that rearing at a lower temperature will enhance fitness at all oviposition temperatures (rearing_(l) is significant with a negative coefficient) whereas the hotter is better hypothesis predicts that rearing at a higher temperature will enhance fitness at all oviposition temperatures (rearing_(l) is significant with a positive coefficient). The optimal developmental temperature hypothesis predicts that rearing at an intermediate temperature will enhance fitness at all oviposition temperatures (rearing_(q) is significant with a negative coefficient). The beneficial acclimation hypothesis predicts that an individual's fitness will be greatest when oviposition temperature and rearing temperature are the same (rearing_(l) \times oviposition and rearing_(q) \times oviposition interactions significant). Although the analysis can indicate significant

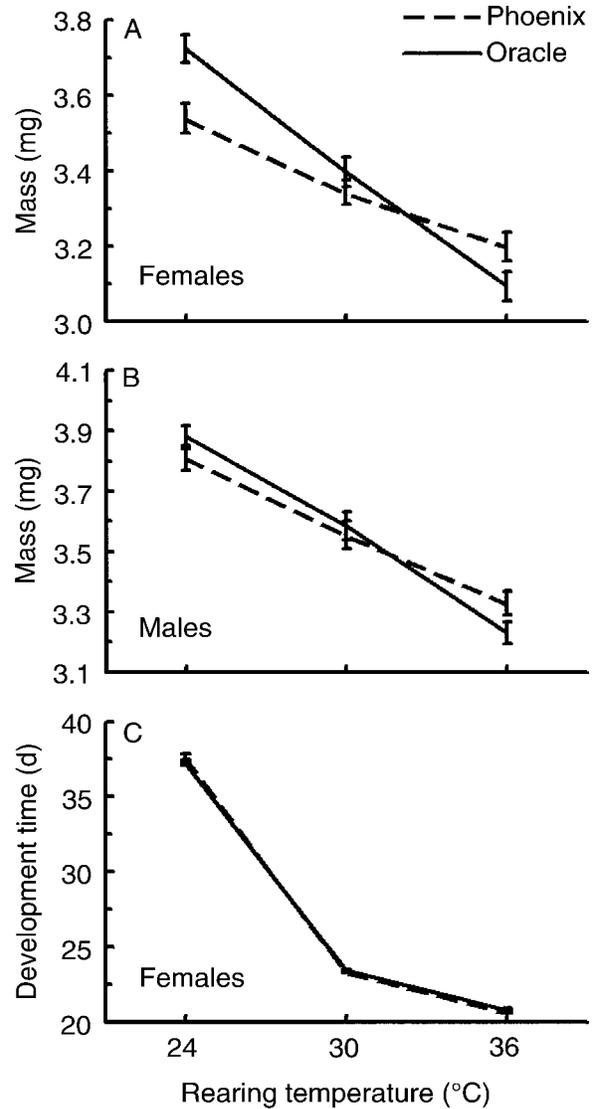


FIG. 1. The effect of rearing temperature on (A) female body mass, (B) male body mass, and (C) female development time of beetles from Phoenix (low-elevation) and Oracle (high-elevation) populations in Arizona, USA (means \pm 1 SE).

support for several hypotheses simultaneously, the relative importance of each can be determined by comparing F ratios. Huey et al. (1999) provides details of the analysis and an extensive discussion of the hypotheses.

RESULTS

Main effects of rearing temperature

Body mass decreased with increasing rearing temperature for both male and female beetles (Fig. 1A, B; Table 1; $P < 0.0001$), consistent with the typical pattern in ectothermic animals (Atkinson 1994). At all rearing temperatures males were larger than females (highly

TABLE 1. ANOVAs (Type III sums of squares) for the effects of rearing temperature and population on adult body mass (including the effect of sex) and female development time of *Stator limbatus* reared from populations found in Arizona, USA.

Source	df	F	P
Mass (mg)			
Rearing temperature (R)	2	178.02	<0.0001
Population (P)	1	0.92	0.34
Sex (S)	1	65.10	<0.0001
R × P	2	8.56	0.0002
R × S	2	1.21	0.30
P × S	1	0.95	0.33
R × P × S	2	0.62	0.54
Error	997		
Female development time (d)			
R	2	10 279.40	<0.0001
P	1	0.96	0.33
R × P	2	3.37	0.04
Error	496		

significant sex effect; Table 1; $P < 0.0001$) as observed in other studies of *Stator limbatus* (Savalli and Fox 1998). Body size (of both sexes) decreased ~6–9% between 24° and 30°C and decreased ~4–10% between 30° and 36°C. Beetles took longer to develop at lower temperatures (Fig. 1C; Table 1; $P < 0.0001$; data collected for females only).

Age-at-first-reproduction (measured as time between emergence and first oviposition) was lowest at 30°C (Fig. 2A, B; Table 2; $P < 0.0001$). Female lifetime fecundity decreased monotonically with increasing rearing temperature (Fig. 2C, D; Table 2; $P < 0.0001$). Interestingly, females reared at 30°C (the intermediate temperature) laid the smallest eggs (vs. those raised at 24°C and 30°C; Fig. 2E, F; Table 2; $P = 0.01$), which is not the monotonic relationship (larger eggs at lower temperatures) found for most arthropods (Fox and Czesak 2000).

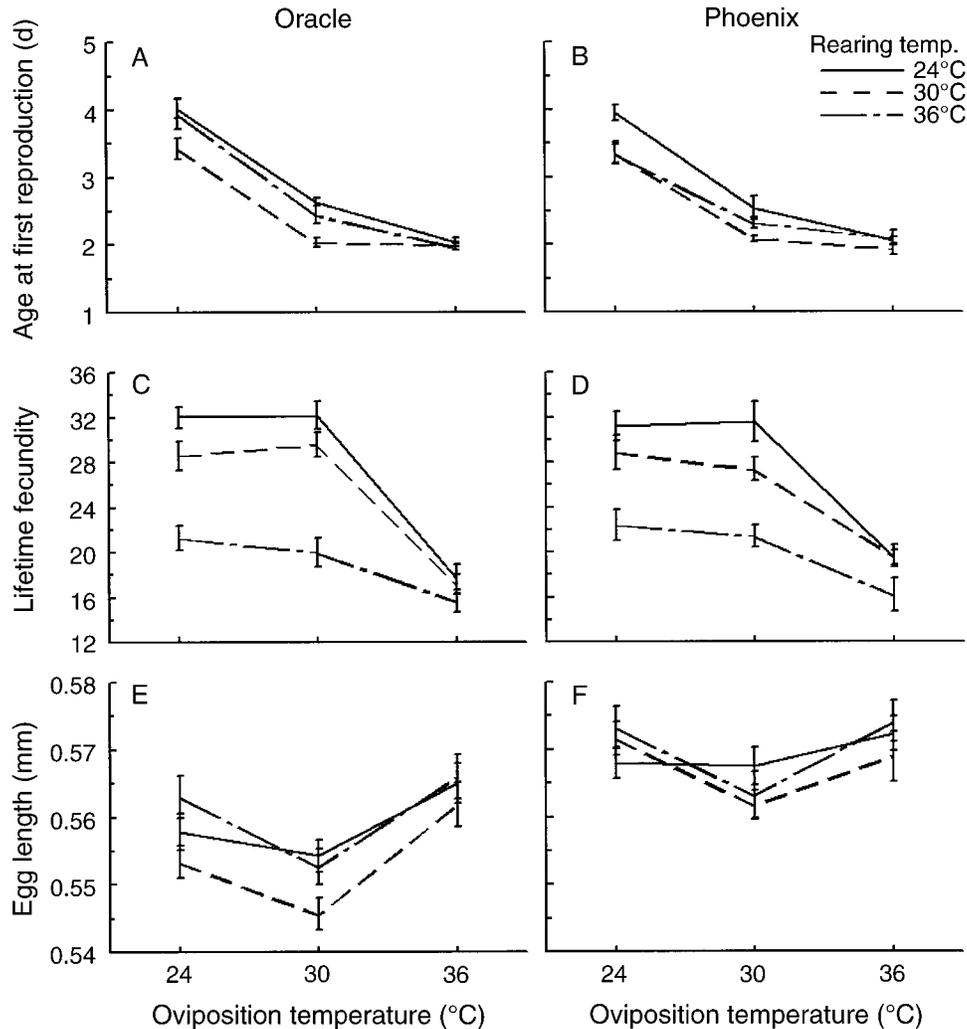


FIG. 2. The effect of both rearing temperature and oviposition temperature on (A, B) age-at-first-reproduction, (C, D) lifetime fecundity, and (E, F) egg length of females from the (A, C, E) Oracle and (B, D, F) Phoenix populations (means \pm 1 SE).

TABLE 2. ANOVAs (Type III sums of squares) for the effects of rearing temperature, oviposition temperature, and population on life history traits of *Stator limbatus*.

Source	df	F	P
Age at first reproduction (d)			
Rearing temperature (R)	2	16.58	<0.0001
Oviposition temperature (O)	2	307.46	<0.0001
Population (P)	1	2.8	0.09
R × O	4	2.38	0.05
R × P	2	0.89	0.41
O × P	2	1.93	0.15
R × O × P	4	1.29	0.27
Error	478		
Lifetime fecundity			
R	2	64.38	<0.0001
O	2	114.84	<0.0001
P	1	0.55	0.46
R × O	4	6.51	<0.0001
R × P	2	0.24	0.79
O × P	2	1.11	0.33
R × O × P	4	0.72	0.58
Error	470		
Egg length (mm)			
R	2	4.7	0.01
O	2	21.26	<0.0001
P	1	68.26	<0.0001
R × O	4	1.23	0.30
R × P	2	0.94	0.39
O × P	2	1.81	0.17
R × O × P	4	0.43	0.79
Error	463		

Overall, females raised at 36°C laid eggs that were less likely to develop than were eggs laid by females raised at 24° and 30°C (Fig. 3A, B; $\chi^2_2 = 5.73$, $P = 0.06$). There was no effect of rearing temperature on egg hatching (Fig. 3C, D; $\chi^2_2 = 1.90$, $P = 0.39$); but see the interaction results discussed below). In general, r_{C1} (the first composite measure of fitness) decreased with increased rearing temperature (Fig. 4A, B; Table 3; $P < 0.001$) while r_{C2} (the second composite measure of fitness that considers age-at-first-reproduction) was greatest at 30°C (Fig. 4C, D; Table 3; $P < 0.001$).

Main effects of oviposition temperature

Oviposition temperature had a larger effect than did rearing temperature on age-at-first-reproduction, fecundity, and egg size (comparison of F ratios in Table 2). Females took longer to initiate reproduction at lower oviposition temperatures (Fig. 2A, B; Table 2; $P < 0.0001$). Fecundity decreased sharply from 30° to 36°C, but there were only small differences between 24° and 30°C (Fig. 2C, D; Table 2; $P < 0.0001$). Females also laid the smallest eggs when they oviposited at 30°C (Fig. 2E, F; Table 2; $P < 0.0001$), which was similar to the effect of rearing temperature on egg size. There was no overall effect of oviposition temperature on either egg development (Fig. 3A, B; $\chi^2_2 = 0.50$, $P = 0.78$) or egg hatching (Fig. 3C, D; $\chi^2_2 = 0.42$, $P = 0.81$) (but see the interaction results discussed below). r_{C1} decreased with increasing oviposition temperature

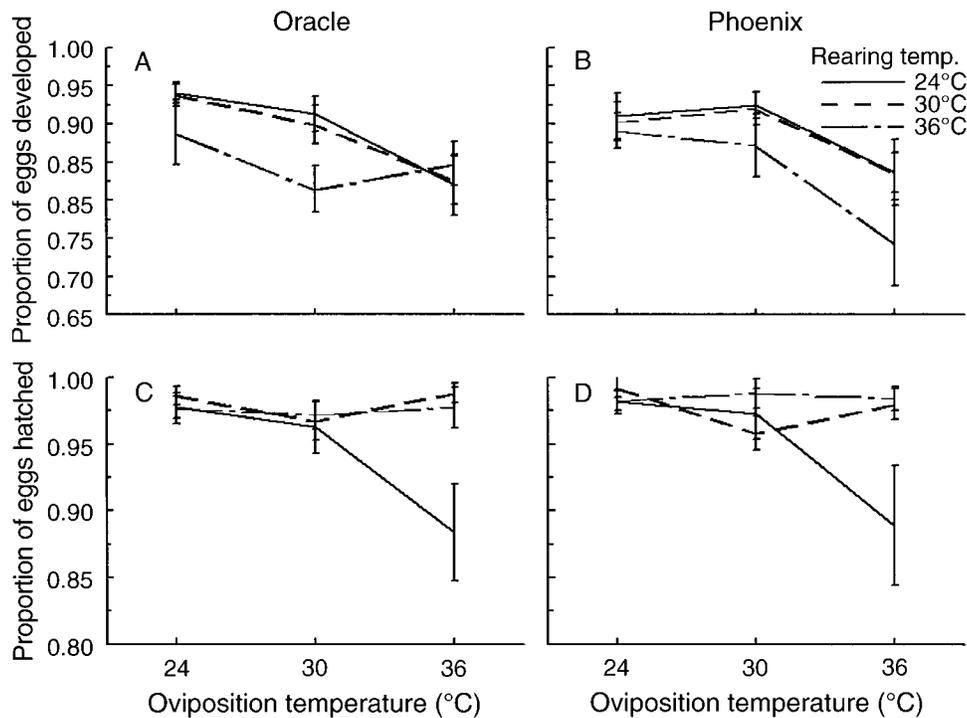


FIG. 3. The effect of both rearing temperature and oviposition temperature on (A, B) proportion of eggs that developed into an embryo and (C, D) proportion of those that developed that hatched, for eggs laid by female beetles from (A, C) Oracle and (B, D) Phoenix populations (means ± 1 SE).

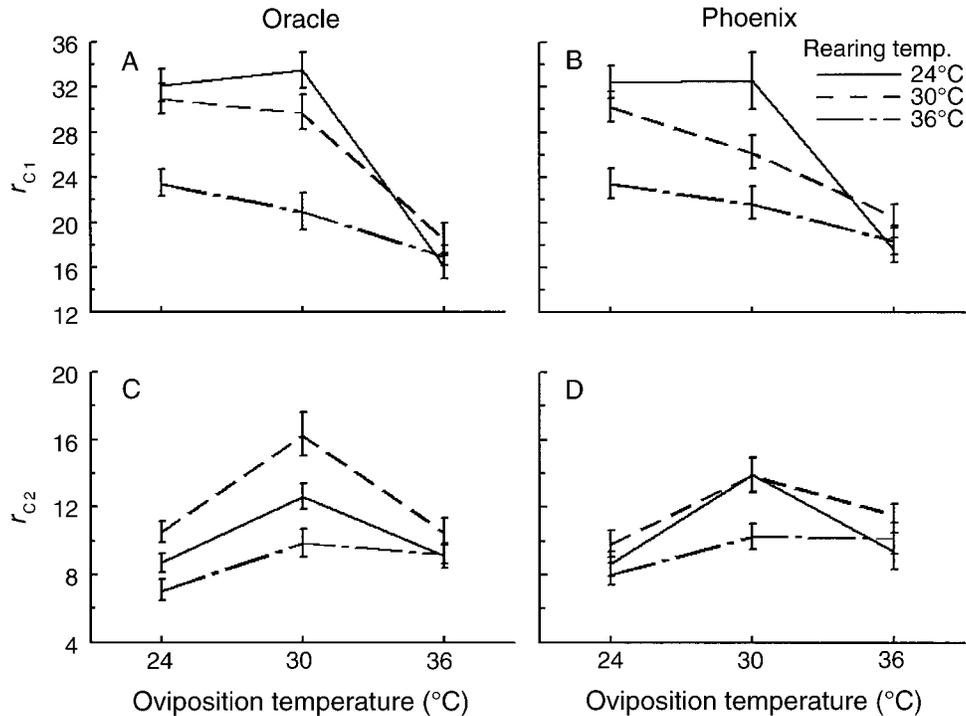


FIG. 4. The effect of both rearing temperature and oviposition temperature on the composite measures of fitness, (A, B) r_{C1} and (C, D) r_{C2} , for beetles from (A, C) Oracle and (B, D) Phoenix populations (means \pm 1 SE).

(Fig. 4A, B; Table 3; $P < 0.001$) but r_{C2} was highest at the intermediate oviposition temperature (Fig. 4C, D; Table 3; $P < 0.01$).

Main effects of population and population \times temperature interactions

When raised at 24°C, Oracle beetles were larger than Phoenix beetles, whereas Phoenix beetles were larger than Oracle beetles when raised at 36°C (significant rearing temperature \times population interaction; Fig. 1A, B; Table 1; $P = 0.0002$). Likewise, Oracle females matured sooner than Phoenix beetles when raised at 24°C, whereas Phoenix beetles matured sooner at 36°C, though the effect was very small (significant rearing temperature \times population interaction; Fig. 1C; Table 1; $P = 0.04$). Both of these results are consistent with our expectation that Oracle beetles are adapted to lower temperatures and Phoenix beetles are adapted to higher temperatures.

We also predicted that these two populations would be genetically differentiated in several life history traits. In particular, because most ectothermic animals evolve to lay larger eggs in colder environments (e.g., Berven 1982, Azevedo et al. 1996), we expected the Phoenix population to lay more and smaller eggs (due to a trade-off between these two traits; Smith and Fretwell 1974) than the Oracle population. There was no significant difference in mean fecundity between populations (Table 2; $P = 0.46$) but contrary to our pre-

diction, females from the Phoenix population laid larger eggs than females from the Oracle population (Table 2; $P < 0.0001$; least-square means \pm 1 SE; Oracle, 0.55778 ± 0.00095 mm; Phoenix, 0.56896 ± 0.00096 mm).

Rearing temperature \times oviposition temperature interactions

There were interactions between rearing temperature and oviposition temperature for age-at-first-reproduction (Fig. 2A, B; Table 2; $P = 0.05$) and lifetime fecundity (Fig. 2C, D; Table 2; $P < 0.0001$). For example, Phoenix females that were raised at 36°C reached reproductive maturity later than Phoenix females raised at 30°C except when they laid eggs at 24°C (Fig. 2B). Likewise, females raised at 24°C laid more eggs than females raised at 30° and 36°C but Phoenix females raised at 30°C actually laid slightly more eggs at 36°C than Phoenix females raised at 24° or 36°C (Fig. 2D). We also detected significant interactions between rearing temperature and oviposition temperature for egg development (Fig. 3A, B; $\chi^2_4 = 10.84$, $P = 0.03$) and egg hatching (Fig. 3C, D; $\chi^2_4 = 16.01$, $P = 0.003$) indicating large maternal effects. The most dramatic maternal effect was on egg hatching; when females were raised at 24°C they produced eggs that hatched very poorly at 36°C suggesting that maternal acclimation to low temperature comes with a substantial fitness cost if they need to lay eggs at high temperature.

TABLE 3. ANOVAs (Type III sums of squares) with orthogonal polynomial contrasts for the composite measures of fitness, r_{C1} and r_{C2} .

Source	Oracle		Phoenix	
	df	F	df	F
r_{C1}				
Rearing temperature (R)	2	69.65***	2	36.89***
Oviposition temperature (O)	2	22.31***	2	14.11***
R × O	4	6.66***	4	5.07***
R _(l)	1	37.30***	1	26.60***
R _(q)	1	7.33**	1	1.62
O × R _(l)	2	13.21***	2	8.99***
O × R _(q)	2	0.11	2	1.15
Error	144		144	
r_{C2}				
R	2	23.90***	2	15.21***
O	2	17.53***	2	5.39**
R × O	4	2.73*	4	1.74
R _(l)	1	4.91*	1	2.78†
R _(q)	1	30.15***	1	8.00**
O × R _(l)	2	1.67	2	3.45*
O × R _(q)	2	3.79*	2	0.03
Error	144		144	

Note: Abbreviations: l, linear; q, quadratic.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, † $P < 0.10$.

The maternal effect on egg hatching is not mediated through egg size (interaction effect in logistic regression when egg size is included as a covariate; $\chi^2_4 = 15.92$, $P = 0.0031$) indicating that females are influencing egg hatching through egg composition. Also, females raised at 24°C had higher fitness (r_{C1}) when ovipositing at 24° and 30°C, but lower fitness when ovipositing at 36°C compared to females raised at 30° and 36°C (highly significant rearing temperature × oviposition temperature interaction; Fig. 4A, B; Table 3; $P < 0.001$). There was also a suggestion of an interaction for r_{C2} (Table 3; Oracle only, $P < 0.05$); females that were raised at both 24° and 30°C had higher fitness than females raised at 36°C, but differences between females raised at all three rearing temperatures was small when they oviposited at 36°C (Fig. 4C).

Acclimation

For r_{C1} , the colder is better hypothesis was more strongly supported than the other hypotheses (negative rearing_(l), Table 3; Oracle, $F_{1,144} = 37.30$, $P < 0.001$; Phoenix, $F_{1,144} = 26.60$, $P < 0.001$); females raised at 24°C had higher fitness at all oviposition temperatures (except 36°C) than those raised at 30° and 36°C (Fig. 4A, B). Even though females developing at lower temperature performed better overall, the relationship between fitness and temperature was nonlinear for Oracle females (negative rearing_(q), Table 3; $F_{1,144} = 7.33$, $P < 0.01$), suggesting that development at an intermediate temperature (optimal developmental temperature hypothesis) also enhances fitness. There was some support for the beneficial acclimation hypothesis (oviposition × rearing_(l), Table 3; Oracle, $F_{2,144} = 13.21$, $P < 0.001$; Phoenix, $F_{2,144} = 8.99$, $P < 0.001$). In contrast, for r_{C2} the optimal developmental temperature

hypothesis was the most strongly supported of the acclimation hypotheses (negative rearing_(q), Table 3; Oracle, $F_{1,144} = 30.15$, $P < 0.001$; Phoenix, $F_{1,144} = 8.00$, $P < 0.01$); females that developed at 30°C have higher fitness at all oviposition temperatures over females developing at 24° and 36°C (Fig. 4C, D) due to the large effect of temperature on age-at-first-reproduction. However, there was a large negative linear effect of rearing temperature (Table 3; Oracle, $F_{1,144} = 4.91$, $P < 0.05$; Phoenix, $F_{1,144} = 2.78$, $P < 0.10$) and a significant interaction with oviposition temperature (Table 3; Oracle, oviposition × rearing_(q), $F_{2,144} = 3.79$, $P < 0.05$; Phoenix, oviposition × rearing_(l), $F_{2,144} = 3.45$, $P < 0.05$), indicating that, although intermediate temperature was best, fitness dropped off fastest as temperature increased (i.e., colder is better hypothesis) and there was some beneficial effect of acclimation (beneficial acclimation hypothesis).

DISCUSSION

Both rearing and oviposition temperature strongly affected growth and life history traits in the seed beetle, *Stator limbatus*. The rearing and oviposition temperature effects were not always additive, nor in the same direction, and thus reaction norms were highly complex. These complex patterns are important because immature insects are usually subject to different thermal environments than adults. Rearing at a cold (r_{C1} ; colder is better hypothesis) or intermediate (r_{C2} ; optimal developmental temperature hypothesis) temperature enhanced fitness at several adult oviposition temperatures; acclimation had only a small effect on fitness (beneficial acclimation hypothesis). Furthermore, growth traits of the Oracle (high-elevation) and Phoenix (low-elevation) populations responded differently

to rearing temperature providing evidence that populations of *S. limbatus* are differentially adapted to temperature.

Adaptation to temperature

Studies of fruit flies have shown that populations sampled from warm and cool climates exhibit population \times temperature interactions for survivorship and development time (Norry et al. 2001, Bochdanovits and de Jong 2003), suggesting that these populations have adapted to their native climates. We found that the higher elevation population (Oracle) of *S. limbatus* was larger and matured sooner than the lower elevation population (Phoenix) when raised in the colder environment (24°C) while the reverse was true in the hotter environment (36°C), consistent with the hypothesis that these populations are differentially adapted to temperature. Van der Have and de Jong (1996) argued that genotype \times temperature (and by extension population \times temperature) interactions can be due to differential thermal sensitivity of enzymes that control cellular growth and differentiation. Because the Oracle and Phoenix populations experience different thermal environments, it is likely that enzymes that control growth and differentiation have evolved different thermal sensitivities, generating the variation in body size and development time we found among populations. However, further work is needed to reveal the mechanisms that are responsible for producing these types of patterns.

Females from the Phoenix population laid larger eggs than females from the Oracle population. This contradicted our prediction that the higher elevation population should produce larger eggs and is opposite to patterns observed in other species, but is most likely due to host plant adaptation. Natural selection favors larger eggs when *S. limbatus* females lay on *Parkinsonia florida*, and populations collected from *P. florida* have evolved larger eggs than populations collected from *Acacia greggii* (Fox and Mousseau 1996, Fox et al. 2001). That these populations were collected from different host plants (Oracle, *A. greggii*; Phoenix, *P. florida*) possibly confounds interpretation of population differences but cannot account for the population \times temperature interaction we observed for body size and development time. If population differences are due to host plant adaptation, then we should have observed differences between the populations across all temperatures as was demonstrated with egg size.

Complex patterns of phenotypic plasticity

Interactions between temperatures experienced in separate stages of the life cycle can dramatically affect reaction norm shape (Gibert et al. 2001). Because natural selection will act on the entire reaction norm produced by the total effect of temperature experienced throughout an individual's life, it is important to know whether these effects are additive or nonadditive and

how reaction norm shape is affected by experiences at different stages of the life cycle. In this study, we showed that temperature-mediated plasticity of age-at-first-reproduction, fecundity, egg development, egg hatching, and two composite measures of fitness were affected by interactions between rearing temperature and oviposition temperature. This is particularly important since larvae experience very different thermal environments than adults; *S. limbatus* larvae are restricted to individual seeds while adults can move from host to host. Such nonadditive effects of temperature on traits closely associated with fitness will complicate efforts to predict evolutionary response to selection (Gibert et al. 2001).

The interaction between rearing temperature and oviposition temperature affected not only a female's phenotype (age-at-first-reproduction and fecundity) but also the survivorship of her offspring in early stages of development (egg development and egg hatching). Females raised at low temperature produced eggs that had substantially reduced hatching when those females oviposited at high temperature (Fig. 3C, D). This large maternal effect on egg hatching was not due to an effect of egg size, implying that females reared at colder temperatures are altering egg composition in such a way that offspring survival is compromised at hotter temperatures. Alternatively, cold-acclimated males may produce sperm that are of lower quality at hotter temperatures. Our study cannot distinguish a paternal effect from a maternal effect. Temperature-mediated parental effects influence offspring fitness in *Drosophila*. For instance, parents raised at higher temperatures produced offspring that had higher fitness than offspring from parents raised at a low or intermediate temperature (Gilchrist and Huey 2001). Also, male flies whose parents had developed at a higher temperature had improved territorial success over males whose parents had been raised at a lower temperature (Zamudio et al. 1995). Though they are difficult to interpret due to their complex nature, temperature-mediated cross-generational effects are likely to be important in the evolution of life histories (Mousseau and Fox 1998). Therefore, future studies should concentrate on the dynamic and complex nature of cross-generation effects of temperature.

The adaptive significance of acclimation

Acclimation is assumed to be beneficial and ubiquitous in organisms, but most empirical tests of the beneficial acclimation hypothesis have challenged this generality (Leroi et al. 1994, Huey et al. 1999, Gibert et al. 2001). Several studies have supported alternative hypotheses such as the optimal developmental temperature hypothesis (Huey et al. 1999, Gibert et al. 2001). These studies have been criticized as having evaluated the adaptive significance of developmental plasticity (irreversible as well as reversible changes to the phenotype induced during development) instead of

traditionally studied acclimation (reversible changes to the phenotype induced in later stages of the life history) and thus are not direct tests of the beneficial acclimation hypothesis (Wilson and Franklin 2002). Although the distinction between developmental plasticity and traditional acclimation is important, focusing too narrowly on the life stage in which plastic responses are induced may obscure the ultimate goal of understanding whether environmentally induced changes to the phenotype are adaptive (Woods and Harrison 2002, Fischer et al. 2003b).

Our results showed that larvae that had developed at a lower (colder is better hypothesis) or intermediate (optimal developmental hypothesis) temperature had the greatest fitness as adults. There was some evidence to support the beneficial acclimation hypothesis but in no case was the beneficial acclimation hypothesis ranked highest among the hypotheses. Therefore, this study is in agreement with other recent empirical analyses suggesting that acclimation has only a small effect on fitness (Leroi et al. 1994, Huey et al. 1999, Gibert et al. 2001). Our rejection of the beneficial acclimation hypothesis is not unexpected because the temperatures that larvae experience within seeds are unlikely to be reliable cues for thermal conditions they will experience as adults, which may prevent the evolution of adaptive acclimation. Alternatively, selection may favor physiologically robust individuals that have the ability to perform well in a variety of adult thermal environments (Gibert et al. 2001). Our analyses were based on indirect estimates of fitness and should thus be interpreted with caution. However, a study that measured fitness directly showed that bacteria acclimated to 41.5°C had reduced fitness when tested at 41.5°C compared to bacteria that had been acclimated to 32°C, demonstrating that acclimation is not beneficial (Leroi et al. 1994).

We based our test of the beneficial acclimation hypothesis on estimates of fitness but Woods and Harrison (2002) argue that acclimation can be better understood by exploring responses of individual traits. When the life history traits were evaluated individually, the hypothesis that was best supported by our data varied among traits (colder is better [fecundity, egg development], hotter is better [egg hatching], and optimal developmental temperature [age-at-first-reproduction]; no hypothesis was supported by egg size) but there was no support for the beneficial acclimation hypothesis. Therefore, an evaluation of acclimation based solely on individual traits is largely uninterpretable because developmental temperature can simultaneously alter a large number of traits in opposite directions. The best solution may be to simultaneously explore the responses of multiple traits, the relationships between those traits, and their consequences for fitness.

Temperatures used in experimental designs of acclimation studies can have substantial implications for conclusions when assessing the beneficial acclimation

hypothesis. For example, development at stressful temperatures can severely impact fitness, overwhelming any beneficial effects of acclimation (Wilson and Franklin 2002). Likewise, development at constant temperatures (as well as chronic exposure to any environmental factor) may be stressful for organisms and could lead to incorrect evaluations of the beneficial acclimation hypothesis (Woods and Harrison 2002). The temperatures used in this study were selected because they are within the normal range of temperatures encountered in the field but inclusion of other temperatures and variation in temperature within treatments could produce different results.

Conclusions

Responses of growth traits of low- and high-elevation populations of the seed beetle, *Stator limbatus*, to rearing temperature indicate that these populations have adapted to their local thermal environments. In addition, beetles developing at a low (colder is better hypothesis) or intermediate temperature (optimal developmental temperature hypothesis) had large fitness advantages as adults over beetles that developed at a hotter temperature; there was little evidence that acclimation is adaptive (beneficial acclimation hypothesis). Most importantly, we detected strong interactions between the effects of rearing and oviposition temperature on multiple traits, producing complex reaction norms and indicating that reaction norm shape is affected by nonadditive effects of temperature experienced across life stages. Complexity in plasticity will make it difficult to predict evolutionary responses to natural selection, but an understanding of this complexity should yield new insights into the adaptive significance of plasticity itself.

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